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(11) Publication number: **0 527 628 A1**

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: **92307307.6**

(51) Int. Cl.⁵: **C12Q 1/68**, C07H 21/00,
C12Q 1/14, // (C12Q1/14,
C12R1:445, 1:45)

(22) Date of filing: **10.08.92**

(30) Priority: **13.08.91 US 744770**

(43) Date of publication of application:
17.02.93 Bulletin 93/07

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU NL
PT SE**

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(54) **Rapid method for detection of methicillin-resistant staphylococci.**

(57) The present invention provides a method for the detection of methicillin-resistant staphylococci using the polymerase chain reaction. The reaction detects the presence of the *mecA* gene, which encodes penicillin binding protein 2A. Also provided is a method for the rapid release of DNA from staphylococci. The two methods can be used in combination for a rapid and sensitive route to the detection of these dangerous pathogens.

0 527 628 A1

Methicillin resistance in staphylococci is an important universal problem in hospitals and geriatric care centers. Both methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus epidermidis (MRSE) are common nosocomial pathogens. The infections they cause are serious and difficult to treat. Horan et al., Morbidity Mortality Weekly Rep. 35:1755 (1984); Maple et al., Lancet i:537 (1989). Only a few antibiotics are available for treatment and they all have undesirable side effects. Hackbarth and Chambers, Antimicrob. Agents Chemother. 33:995 (1989). Currently, time-consuming, labor intensive, and somewhat unreliable methods are employed for the detection of MRSA/MRSE. Id These methods include disk diffusion, broth dilution and agar screening. Id Such methods do not reliably detect heterogeneously resistant staphylococci. Thus, it is imperative to develop a rapid, standardized, accurate and sensitive method for the detection of methicillin resistance in staphylococci.

MRSA/MRSE carry the mecA gene which encodes penicillin binding protein 2a (PBP2a). This proteins responsible for the phenotypic expression of methicillin resistance in staphylococci. Chambers, Antimicrob. Agents Chemother. 33:424 (1989); Hackbarth and Chambers, Antimicrob. Agents Chemother. 33:991 (1989); Tonin and Tomasz, Antimicrob. Agents Chemother. 30:577 (1986). Staphylococcal strains susceptible to methicillin do not harbor a mecA gene. Therefore, the mecA gene is a useful molecular handle for rapid identification of MRSA/MRSE. Detection of mecA by DNA hybridization has provided a relatively sensitive method for identifying MRSA/MRSE strains. Archer and Penell, Antimicrob. Agents Chemother. 34:1720 (1990); Lencastre et al., Antimicrob. Agents Chemother. 35:575 (1991). However, DNA hybridization suffers from several disadvantages. A large number of cells are required, DNA extraction and immobilization on a membrane is a time-consuming process, and frequently radioactive isotopes are employed, although recently non-radioactive probes have become available. Ligozzi et al., Antimicrob. Agents Chemother. 35:575 (1991).

The present invention provides a method for detecting methicillin-resistant staphylococcal infections, said method comprising:

- a) performing the polymerase chain reaction on clinical samples suspected of staphylococcal infection, said polymerase chain reaction being primed by DNA primers composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
- b) analyzing the reaction product of step a.

The present invention also provides a method for the rapid release of DNA from staphylococci, said method comprising:

- a) treating a sample containing staphylococci with lysostaphin;
- b) treating the resultant sample of step a with proteinase K; and
- c) incubating the resultant sample of step b in a boiling water bath.

Additionally, the present invention provides a method for detecting methicillin-resistant staphylococcal infections in a sample of interest, said method comprising:

- a) treating a sample of interest with lysostaphin;
- b) treating the resultant sample of step a with proteinase K;
- c) incubating the resultant sample of step b in a boiling water bath;
- d) performing the polymerase chain reaction on the resultant sample of step c, said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
- e) analyzing the reaction product of step d.

For purposes of the present invention the following terms are defined below:

High G+C content - G+C content significantly higher than the average 30.5% G+C content of staphylococcal mecA genes.

mecA gene - a gene encoding penicillin binding protein 2A, which is responsible for methicillin resistance.

Methicillin-resistant staphylococci - staphylococci which are resistant to all beta-lactams, including cephalosporins and penicillin derivatives such as methicillin and oxacillin.

PBP2A - penicillin binding protein 2A.

The present invention provides a method for detecting methicillin-resistant staphylococcal infections, said method comprising:

- a) performing the polymerase chain reaction on clinical samples suspected of staphylococcal infection, said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised

by the non-coding strand of a Staphylococcus mecA gene; and

b) analyzing the reaction product of step a.

The polymerase chain reaction (PCR) is now well-known in the art as a route to the amplification of minute quantities of DNA. See, U.S. Patents 4,683,195, 4,800,159 and 4,683,202. The technique has been applied in clinical settings to the detection of a variety of different pathogens. See, e.g., Cassol et al., J. Clin. Microbiol. 29:667-671 (1991); Lopez et al., J. Clin. Microbiol. 29:578-582 (1991); Brisson-Noel et al., Lancet ii:1069-1071 (1989); Valentine et al., J. Clin. Microbiol. 29:689-695 (1991); and Gouvea et al., J. Clin. Microbiol. 29:529-523 (1991). The technique has been used to detect toxins of Staphylococcus aureus but until the present invention the technique was not applied to the detection methicillin-resistant staphylococci.

Staphylococci resistant to beta-lactams, particularly Staphylococcus aureus and Staphylococcus epidermidis, represent a significant and steadily increasing problem for physicians. Due to the speed with which these bacteria can cause death, it is advantageous to quickly ascertain whether the organisms present in a patient are non-responsive to betalactams. The present invention provides a method which can be performed significantly faster than prior art methods (Hackbarth and Chambers, Antimicrob. Agents and Chemother. 33:995-999 (1989)), including methods based on DNA hybridization (Ligozzi et al., Antimicrob. Agents and Chemother. 35:575-578 (1991) and Archer and Pennel, Antimicrob. Agents and Chemother. 34:1720-1724 (1990)). The extreme sensitivity afforded by the PCR is another vital aspect of the present invention. The methicillin-resistant staphylococci are open heterogeneously resistant. Thus, non-DNA based resistance determinations can give inconsistent results.

Penicillin binding protein 2A confers methicillin resistance by having a low affinity for the beta-lactams. The proteins encoded by the mecA gene. The present invention will enable the detection of any staphylococci bearing the mecA gene. The clinically relevant staphylococcal strains are primarily S. epidermidis and S. aureus and, occasionally, S. haemolyticus. Rarely problematic strains are S. simulans, S. carnosus and S. saprophyticus. All the mecA genes which have been isolated and sequenced have a very high similarity (>99%), thus allowing the use of one or two sets of primers to detect all mecA-containing strains. If primers are made from regions of the gene which are known to contain variance among mecA genes isolated from different sources, the length of the primers used should be at least thirty nucleotides to ensure specific priming. These regions correlate to nucleotides 605-607, 615-617, 697-699, 746-747, 841, 1010-1011, 1819, and 1933 of SEQ ID NO:1.

The average G+C content of the known mecA genes is about 30.5%. To ensure specific priming, the G+C content of the DNA primers used for the PCR should be significantly higher than 30.5%, preferably about 50% or higher. The sequences of the DNA primers may be derived from any mecA gene. The sequences of some of these genes can be found in Song et al., FEBS Letters 221:167-171 (1987) (S. aureus TK784) and Ryffel et al., Gene 94:137-38 (1990) (S. aureus BB270 and S. epidermidis WT55). Additionally, the DNA primers may be derived from the S. aureus 27R mecA gene, set out as SEQ ID NO:1.

Skilled artisans will recognize that, within a particular set of primers, one of the oligonucleotides should be derived from the coding strand of the gene and the other oligonucleotide should be derived from the non-coding strand. Preferred DNA primers have sequences corresponding to nucleotides 141-160 of SEQ ID NO:1 and the inverse complement of nucleotides 1929-1952 of SEQ ID NO:1. The primers can be synthesized by the modified phosphotriester method using fully protected deoxyribonucleotide building blocks. Such synthetic methods are well known in the art and can be carried out in substantial accordance with Itakura et al., Science 198:1056 (1977), Crea et al., Proc. Nat'l Acad. Sci. USA 75:5765 (1978), Hsiung et al., Nuc. Acids Res. 11:3227 (1983) or Narang et al., Methods in Enzymology 68:90, (1980). An especially preferred method employs automated DNA synthesizers such as the Applied Biosystems 380B DNA synthesizer (850 Lincoln Centre Drive, Foster City, CA 94404).

Protocols for performing the PCR reaction are set out in PCR Protocols: A Guide to Methods and Applications, ed. Michael A. Innis et al., Academic Press, Inc., 1990. An especially preferred protocol is described in the Examples herein. The results of the reaction may be analyzed in any way desired; a preferred method is by agarose gel electrophoresis. It may be desirable to further confirm the presence of staphylococci comprising the mecA gene by performing "nested PCR." In this technique, two consecutive PCRs are performed. The oligonucleotides used to prime the second PCR should be derived from DNA sequences of the mecA gene interior to the DNA sequences on which the first set of primers are based. Preferred DNA primers for the second PCR have sequences corresponding to nucleotides 568-593 of SEQ ID NO:1 and the inverse complement nucleotides 1647-1670 of SEQ ID NO:1.

The present invention also provides a method for the rapid release of DNA from staphylococci, said method comprising:

a) treating a sample containing staphylococci with lysostaphin;

b) treating the resultant sample of step a with proteinase K; and

c) incubating the resultant sample of step b in a boiling water bath. The method of the present invention is much faster than prior art staphylococcal DNA extraction methods. A preferred embodiment of the rapid release method is outlined in the Examples. The method is particularly useful in combination with the methicillin-resistant staphylococci detection technique of the present invention. Thus, the present invention also provides a method for detecting methicillin-resistant staphylococcal infections in a sample of interest, said method comprising:

- a) treating a sample interest with lysostaphin;
- b) treating the resultant sample of step a with proteinase K;
- c) incubating the resultant sample of step b in a boiling water bath;
- d) performing the polymerase chain reaction on the resultant sample of step c) said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high G+C content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
- e) analyzing the reaction product of step d.

This method represents a combination of the rapid release DNA extraction method and the method for detecting methicillin-resistant staphylococci outlined above. The various considerations discussed regarding those two techniques are equally applicable when the methods are used in combination. The detection of methicillin-resistant staphylococci via the present invention requires only three hours. In contrast, when the prior art DNA extraction method is used in lieu of the rapid release DNA extraction method, six hours are required. Any additional time is significant in potential life or death situations such as methicillin-resistant staphylococcal infections. The speed and sensitivity of the present invention allows one to avoid empirical antibiotic therapy, which is fraught with liabilities such as high cost, toxicity factors and resistance development.

The present invention may be used to detect methicillin-resistant staphylococci in any internal body fluid, including blood, urine, spinal fluid and fluid drained from an abscess. If desired, the samples may be cultured and the PCR performed on a sample of the bacteria from the growth medium. The use of the PCR on body fluids is known in the prior art. See, e.g., Cassol et al., *J. Clin. Microbiol.* 29:667-671 (1991); Lopez et al., *J. Clin. Microbiol.* 29:578-582 (1991); and Brisson-Noel et al., *Lancet* ii:1069-1071 (1989).

The following Examples are intended to further illustrate and exemplify, but not limit the scope of, the present invention.

Example 1

A total of 70 staphylococcal strains (33 S. aureus and 37 S. epidermidis) isolated from various clinical settings were examined. Species identification was done with a Staph-Ident™ Diagnostic Kit (Analytab, Sherwood Medical, Plainview, NY). To verify the results obtained with the PCR, all were screened for methicillin resistance by growth on Mueller-Hinton agar (Difco, Detroit, MI) supplemented with 4% sodium chloride and 6 µg oxacillin/ml. Inoculated plates were incubated for 24 hours at 35 °C and examined for the presence of growth. Sixteen of 33 S. aureus and 27 of 37 S. epidermidis were categorized as methicillin-resistant using this method.

Example 2

Each strain examined for methicillin resistance in Example 1, above, was treated as follows. Bacteria were harvested from either TY agar plates (one loopful) or TY broth cultures (one ml of overnight culture diluted to 10⁸ bacteria/ml). Cells from broth medium were harvested by centrifugation for 30 seconds in a microcentrifuge. Cells from either source were resuspended in 50 µl of lysostaphin solution (100 µg/ml in water, Sigma Chemical Co., St. Louis, MO). Cell suspensions were incubated at 37 °C. After 10 minutes, 50 µl proteinase K solution (100 µg/ml in water, Sigma), and 150 µl of 0.1 M Tris, pH 7.5, were added. The cell suspensions were incubated at 37 °C for an additional 20 minutes and then placed in a boiling water bath for 10 minutes. This treatment effectively lysed S. aureus or S. epidermidis cells and prevented DNase activity. Ten µl from these cell lysates were used directly in PCRs.

Example 3

Two primers were chosen for the PCR that were separated by 1.8 kb in the mecA open reading frame. The sequences of the two primers were 5'-GTTGTAGTTGTCGGGTTTGG-3' (nucleotides 141-160 of SEQ ID NO:1) and 5'-CCACCCAATTTGTCTGCCAGTTTCTCC-3' (inverse complement of nucleotides 1929-1952 of SEQ ID NO:1). The PCR was performed in a DNA Thermal Cycler using a Gene Amp Kit according to the manufacturer's

instructions (Perkin Elmer Cetus, Norwalk, CT). A thermal step program that included the following parameters was used for DNA amplification: denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, primer extension at 72 °C for two minutes, for a total of 30 cycles. Ten µl of the PCR solution were analyzed by electrophoresis on a 0.8% agarose gel. A positive result was indicated by the presence of a 1.8 kb amplified DNA fragment. In all, 69 of the 70 staphylococcal strains produced exactly the same result with PCR and the oxacillin susceptibility test. Examination of the single exception revealed that it was a mixed culture containing a rapidly growing methicillin-sensitive strain (mecA⁻) and a slower growing methicillin-resistant (mecA⁺) strain. When the strains were separated the susceptibility test and the PCR were in agreement. Subsequent nested PCR analysis of the initial DNA sample with the internal DNA primers (corresponding to nucleotides 568-593 of SEQ ID NO:1 and the inverse complement of nucleotides 1647-1670 of SEQ ID NO:1) produced a positive PCR result. No isolates were found to be mecA sensitive but methicillin resistant. These results emphasize the fact that a positive result in a DNA-based test correlates very well with the methicillin-resistant phenotype.

SEQUENCE LISTING

5

10

(1) GENERAL INFORMATION:

15

- (i) APPLICANT: Eli Lilly and Company
- (ii) TITLE OF INVENTION: Rapid Method for Detection of
Methicillin Resistant Staphylococci
- (iii) NUMBER OF SEQUENCES: 2

20

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25

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 - (C) REFERENCE/DOCKET NUMBER: X-8573

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35

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(2) INFORMATION FOR SEQ ID NO:1:

45

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2111 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

50

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

55

5	(ix) FEATURE:		
	(A) NAME/KEY: CDS		
	(B) LOCATION: 105..2111		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:		
10	ACACCTTCTA CACCTCGATA TCACAAAAAA TTATAACATT ATTTTGACAT AAATACTACA	60	
	TTTGTAAATAT ACTACAAATG TAGTCTTATA TAAGGAGGAT ATTG ATG AAA AAG ATA	116	
	Met Lys Lys Ile		
	1		
15	AAA ATT GTT CCA CTT ATT TTA ATA GTT GTA GTT GTC GGG TTT GGT ATA	164	
	Lys Ile Val Pro Leu Ile Leu Ile Val Val Val Val Gly Phe Gly Ile		
	5 10 15 20		
	TAT TTT TAT GCT TCA AAA GAT AAA GAA ATT AAT AAT ACT ATT GAT GCA	212	
	Tyr Phe Tyr Ala Ser Lys Asp Lys Glu Ile Asn Asn Thr Ile Asp Ala		
20	25 30 35		
	ATT GAA GAT AAA AAT TTC AAA CAA GTT TAT AAA GAT AGC AGT TAT ATT	260	
	Ile Glu Asp Lys Asn Phe Lys Gln Val Tyr Lys Asp Ser Ser Tyr Ile		
	40 45 50		
25	TCT AAA AGC GAT AAT GGT GAA GTA GAA ATG ACT GAA CGT CCG ATA AAA	308	
	Ser Lys Ser Ser Asp Asn Gly Glu Val Glu Met Thr Glu Arg Pro Ile Lys		
	55 60 65		
	ATA TAT AAT AGT TTA GGC GTT AAA GAT ATA AAC ATT CAG GAT CGT AAA	356	
	Ile Tyr Asn Ser Leu Gly Val Lys Asp Ile Asn Ile Gln Asp Arg Lys		
30	70 75 80		
	ATA AAA AAA GTA TCT AAA AAT AAA AAA CGA GTA GAT GCT CAA TAT AAA	404	
	Ile Lys Lys Val Ser Lys Asn Lys Lys Arg Val Asp Ala Gln Tyr Lys		
	85 90 95 100		
35	ATT AAA ACA AAC TAC GGT AAC ATT GAT CGC AAC GTT CAA TTT AAT TTT	452	
	Ile Lys Thr Asn Tyr Gly Asn Ile Asp Arg Asn Val Gln Phe Asn Phe		
	105 110 115		
	GTT AAA GAA GAT GGT ATG TGG AAG TTA GAT TGG GAT CAT AGC GTC ATT	500	
	Val Lys Glu Asp Gly Met Trp Lys Leu Asp Trp Asp His Ser Val Ile		
40	120 125 130		
	ATT CCA GGA ATG CAG AAA GAC CAA AGC ATA CAT ATT GAA AAT TTA AAA	548	
	Ile Pro Gly Met Gln Lys Asp Gln Ser Ile His Ile Glu Asn Leu Lys		
	135 140 145		
45	TCA GAA CGT GGT AAA ATT TTA GAC CGA AAC AAT GTG GAA TTG GCC AAT	596	
	Ser Glu Arg Gly Lys Ile Leu Asp Arg Asn Asn Val Glu Leu Ala Asn		
	150 155 160		
	ACA GGA ACA GCA TAT GAG ATA GGC ATC GTT CCA AAG AAT GTA TCT AAA	644	
	Thr Gly Thr Ala Tyr Glu Ile Gly Ile Val Pro Lys Asn Val Ser Lys		
50			

	165		170		175		180	
5	AAA CAT TAT AAA GCA ATC GCT AAA GAA CTA AGT ATT TCT GAA GAC TAT							692
	Lys Asp Tyr Lys Ala Ile Ala Lys Glu Leu Ser Ile Ser Glu Asp Tyr							
		185			190		195	
10	ATC AAA CAA CAA ATG GAT CAA AAT TGG GTA CAA GAT GAT ACC TTC GTT							740
	Ile Lys Gln Gln Met Asp Gln Asn Trp Val Gln Asp Asp Thr Phe Val							
		200			205		210	
15	CCA CTT AAA ACC GTT AAA AAA ATG GAT GAA TAT TTA AGT GAT TTC GCA							788
	Pro Leu Lys Thr Val Lys Lys Met Asp Glu Tyr Leu Ser Asp Phe Ala							
		215			220		225	
20	AAA AAA TTT CAT CTT ACA ACT AAT GAA ACA GAA AGT CGT AAC TAT CCT							836
	Lys Lys Phe His Leu Thr Thr Asn Glu Thr Glu Ser Arg Asn Tyr Pro							
		230			235		240	
25	CTA GAA AAA GCG ACT TCA CAT CTA TTA GGT TAT GTT GGT CCC ATT AAC							884
	Leu Glu Lys Ala Thr Ser His Leu Leu Gly Tyr Val Gly Pro Ile Asn							
		245			250		255	260
30	TCT GAA GAA TTA AAA CAA AAA GAA TAT AAA GGC TAT AAA GAT GAT GCA							932
	Ser Glu Glu Leu Lys Gln Lys Glu Tyr Lys Gly Tyr Lys Asp Asp Ala							
		265			270		275	
35	GTT ATT GGT AAA AAG GGA CTC GAA AAA CTT TAC GAT AAA AAG CTC CAA							980
	Val Ile Gly Lys Lys Gly Leu Glu Lys Leu Tyr Asp Lys Lys Leu Gln							
		280			285		290	
40	CAT GAA GAT GGC TAT CGT GTC ACA ATC GTT GAC GAT AAT AGC AAT ACA							1028
	His Glu Asp Gly Tyr Arg Val Thr Ile Val Asp Asp Asn Ser Asn Thr							
		295			300		305	
45	ATC GCA CAT ACA TTA ATA GAG AAA AAG AAA AAA GAT GGC AAA GAT ATT							1076
	Ile Ala His Thr Leu Ile Glu Lys Lys Lys Lys Asp Gly Lys Asp Ile							
		310			315		320	
50	CAA CTA ACT ATT GAT GCT AAA GTT CAA AAG AGT ATT TAT AAC AAC ATG							1124
	Gln Leu Thr Ile Asp Ala Lys Val Gln Lys Ser Ile Tyr Asn Asn Met							
		325			330		335	340
55	AAA AAT GAT TAT GGC TCA GGT ACT GCT ATC CAC CCT CAA ACA GGT GAA							1172
	Lys Asn Asp Tyr Gly Ser Gly Thr Ala Ile His Pro Gln Thr Gly Glu							
		345			350		355	
60	TTA TTA GCA CTT GTA AGC ACA CCT TCA TAT GAC GTC TAT CCA TTT ATG							1220
	Leu Leu Ala Leu Val Ser Thr Pro Ser Tyr Asp Val Tyr Pro Phe Met							
		360			365		370	
65	TAT GGC ATG AGT AAC GAA GAA TAT AAT AAA TTA ACC GAA GAT AAA AAA							1268
	Tyr Gly Met Ser Asn Glu Glu Tyr Asn Lys Leu Thr Glu Asp Lys Lys							
		375			380		385	
70	GAA CCT CTG CTC AAC AAG TTC CAG ATT ACA ACT TCA CCA GGT TCA ACT							1316
	Glu Pro Leu Leu Asn Lys Phe Gln Ile Thr Thr Ser Pro Gly Ser Thr							

	390	395	400	
5	CAA AAA ATA TTA ACA GCA ATG ATT GGG TTA AAT AAC AAA ACA TTA GAC Gln Lys Ile Leu Thr Ala Met Ile Gly Leu Asn Asn Lys Thr Leu Asp 405 410 415 420	1364		
10	GAT AAA ACA AGT TAT AAA ATC GAT GGT AAA GGT TGG CAA AAA GAT AAA Asp Lys Thr Ser Tyr Lys Ile Asp Gly Lys Gly Trp Gln Lys Asp Lys 425 430 435	1412		
15	TCT TGG GGT GGT TAC AAC GTT ACA AGA TAT GAA GTG GTA AAT GGT AAT Ser Trp Gly Gly Tyr Asn Val Thr Arg Tyr Glu Val Val Asn Gly Asn 440 445 450	1460		
20	ATC GAC TTA AAA CAA GCA ATA GAA TCA TCA GAT AAC ATT TTC TTT GCT Ile Asp Leu Lys Gln Ala Ile Glu Ser Ser Asp Asn Ile Phe Phe Ala 455 460 465	1508		
25	AGA GTA GCA CTC GAA TTA GGC AGT AAG AAA TTT GAA AAA GGC ATG AAA Arg Val Ala Leu Glu Leu Gly Ser Lys Lys Phe Glu Lys Gly Met Lys 470 475 480	1556		
30	AAA CTA GGT GTT GGT GAA GAT ATA CCA AGT GAT TAT CCA TTT TAT AAT Lys Leu Gly Val Gly Glu Asp Ile Pro Ser Asp Tyr Pro Phe Tyr Asn 485 490 495 500	1604		
35	GCT CAA ATT TCA AAC AAA AAT TTA GAT AAT GAA ATA TTA TTA GCT GAT Ala Gln Ile Ser Asn Lys Asn Leu Asp Asn Glu Ile Leu Leu Ala Asp 505 510 515	1652		
40	TCA GGT TAC CGA CAA GGT CAA ATA CTG ATT AAC CCA GTA CAG ATC CTT Ser Gly Tyr Gly Gln Gly Glu Ile Leu Ile Asn Pro Val Gln Ile Leu 520 525 530	1700		
45	TCA ATC TAT AGC GCA TTA GAA AAT AAT GGC AAT ATT AAC GCA CCT CAC Ser Ile Tyr Ser Ala Leu Glu Asn Asn Gly Asn Ile Asn Ala Pro His 535 540 545	1748		
50	TTA TTA AAA GAC ACG AAA AAC AAA GTT TGG AAG AAA AAT ATT ATT TCC Leu Leu Lys Asp Thr Lys Asn Lys Val Trp Lys Lys Asn Ile Ile Ser 550 555 560	1796		
55	AAA GAA AAT ATC AAT CTA TTA ACT GAT GGT ATG CAA CAA GTC GTA AAT Lys Glu Asn Ile Asn Leu Leu Thr Asp Gly Met Gln Gln Val Val Asn 565 570 575 580	1844		
60	AAA ACA CAT AAA GAA GAT ATT TAT AGA TCT TAT GCA AAC TTA ATT GGC Lys Thr His Lys Glu Asp Ile Tyr Arg Ser Tyr Ala Asn Leu Ile Gly 585 590 595	1892		
65	AAA TCC GGT ACT GCA GAA CTC AAA ATG AAA CAA GGA GAA ACT GGC AGA Lys Ser Gly Thr Ala Glu Leu Lys Met Lys Gln Gly Glu Thr Gly Arg 600 605 610	1940		
70	CAA ATT GGG TGG TTT ATA TCA TAT GAT AAA GAT AAT CCA AAC ATG ATG Gln Ile Gly Trp Phe Ile Ser Tyr Asp Lys Asp Asn Pro Asn Met Met	1988		

5 615 620 625

ATG GCT ATT AAT GTT AAA GAT GTA CAA GAT AAA GGA ATG GCT AGC TAC 2036
Met Ala Ile Asn Val Lys Asp Val Gln Asp Lys Gly Met Ala Ser Tyr
530 635 640

10 645 650 655 660

AAT GCC AAA ATC TCA GGT AAA GTG TAT GAT GAG CTA TAT GAG AAC GGT 2084
Asn Ala Lys Ile Ser Gly Lys Val Tyr Asp Glu Leu Tyr Glu Asn Gly
645 650 655 660

AAT AAA AAA TAC GAT ATA GAT GAA TA 2111
Asn Lys Lys Tyr Asp Ile Asp Glu
665

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 668 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 1 5 10 15

Met Lys Lys Ile Lys Ile Val Pro Leu Ile Leu Ile Val Val Val Val
1 5 10 15

Gly Phe Gly Ile Tyr Phe Tyr Ala Ser Lys Asp Lys Glu Ile Asn Asn
20 25 30

30 35 40 45

Thr Ile Asp Ala Ile Glu Asp Lys Asn Phe Lys Gln Val Tyr Lys Asp
35 40 45

Ser Ser Tyr Ile Ser Lys Ser Asp Asn Gly Glu Val Glu Met Thr Glu
50 55 60

35 65 70 75 80

Arg Pro Ile Lys Ile Tyr Asn Ser Leu Gly Val Lys Asp Ile Asn Ile
65 70 75 80

Gln Asp Arg Lys Ile Lys Lys Val Ser Lys Asn Lys Lys Arg Val Asp
85 90 95

40 100 105 110

Ala Gln Tyr Lys Ile Lys Thr Asn Tyr Gly Asn Ile Asp Arg Asn Val
100 105 110

Gln Phe Asn Phe Val Lys Glu Asp Gly Met Trp Lys Leu Asp Trp Asp
115 120 125

45 130 135 140

His Ser Val Ile Ile Pro Gly Met Gln Lys Asp Gln Ser Ile His Ile
130 135 140

Glu Asn Leu Lys Ser Glu Arg Gly Lys Ile Leu Asp Arg Asn Asn Val
145 150 155 160

50

55

Glu Leu Ala Asn Thr Gly Thr Ala Tyr Glu Ile Gly Ile Val Pro Lys
 165 170 175
 5 Asn Val Ser Lys Lys Asp Tyr Lys Ala Ile Ala Lys Glu Leu Ser Ile
 180 185 190
 Ser Glu Asp Tyr Ile Lys Gln Gln Met Asp Gln Asn Trp Val Gln Asp
 195 200 205
 10 Asp Thr Phe Val Pro Leu Lys Thr Val Lys Lys Met Asp Glu Tyr Leu
 210 215 220
 Ser Asp Phe Ala Lys Lys Phe His Leu Thr Thr Asn Glu Thr Glu Ser
 225 230 235 240
 15 Arg Asn Tyr Pro Leu Glu Lys Ala Thr Ser His Leu Leu Gly Tyr Val
 245 250 255
 Gly Pro Ile Asn Ser Glu Glu Leu Lys Gln Lys Glu Tyr Lys Gly Tyr
 260 265 270
 20 Lys Asp Asp Ala Val Ile Gly Lys Lys Gly Leu Glu Lys Leu Tyr Asp
 275 280 285
 Lys Lys Leu Gln His Glu Asp Gly Tyr Arg Val Thr Ile Val Asp Asp
 290 295 300
 25 Asn Ser Asn Thr Ile Ala His Thr Leu Ile Glu Lys Lys Lys Lys Asp
 305 310 315 320
 Gly Lys Asp Ile Gln Leu Thr Ile Asp Ala Lys Val Gln Lys Ser Ile
 325 330 335
 30 Tyr Asn Asn Met Lys Asn Asp Tyr Gly Ser Gly Thr Ala Ile His Pro
 340 345 350
 Gln Thr Gly Glu Leu Leu Ala Leu Val Ser Thr Pro Ser Tyr Asp Val
 355 360 365
 35 Tyr Pro Phe Met Tyr Gly Met Ser Asn Glu Glu Tyr Asn Lys Leu Thr
 370 375 380
 40 Glu Asp Lys Lys Glu Pro Leu Leu Asn Lys Phe Gln Ile Thr Thr Ser
 385 390 395 400
 Pro Gly Ser Thr Gln Lys Ile Leu Thr Ala Met Ile Gly Leu Asn Asn
 405 410 415
 45 Lys Thr Leu Asp Asp Lys Thr Ser Tyr Lys Ile Asp Gly Lys Gly Trp
 420 425 430
 Gln Lys Asp Lys Ser Trp Gly Gly Tyr Asn Val Thr Arg Tyr Glu Val
 435 440 445
 50 Val Asn Gly Asn Ile Asp Leu Lys Gln Ala Ile Glu Ser Ser Asp Asn
 450 455 460

Ile Phe Phe Ala Arg Val Ala Leu Glu Leu Gly Ser Lys Lys Phe Glu
 465 470 475 480
 5 Lys Gly Met Lys Lys Leu Gly Val Gly Glu Asp Ile Pro Ser Asp Tyr
 485 490 495
 Pro Phe Tyr Asn Ala Gln Ile Ser Asn Lys Asn Leu Asp Asn Glu Ile
 500 505 510
 10 Leu Leu Ala Asp Ser Gly Tyr Gly Gln Gly Glu Ile Leu Ile Asn Pro
 515 520 525
 Val Gln Ile Leu Ser Ile Tyr Ser Ala Leu Glu Asn Asn Gly Asn Ile
 530 535 540
 15 Asn Ala Pro His Leu Leu Lys Asp Thr Lys Asn Lys Val Trp Lys Lys
 545 550 555 560
 Asn Ile Ile Ser Lys Glu Asn Ile Asn Leu Leu Thr Asp Gly Met Gln
 565 570 575
 20 Gln Val Val Asn Lys Thr His Lys Glu Asp Ile Tyr Arg Ser Tyr Ala
 580 585 590
 Asn Leu Ile Gly Lys Ser Gly Thr Ala Glu Leu Lys Met Lys Gln Gly
 595 600 605
 25 Glu Thr Gly Arg Gln Ile Gly Trp Phe Ile Ser Tyr Asp Lys Asp Asn
 610 615 620
 Pro Asn Met Met Met Ala Ile Asn Val Lys Asp Val Gln Asp Lys Gly
 625 630 635 640
 30 Met Ala Ser Tyr Asn Ala Lys Ile Ser Gly Lys Val Tyr Asp Glu Leu
 645 650 655
 35 Tyr Glu Asn Gly Asn Lys Lys Tyr Asp Ile Asp Glu
 660 665

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Claims

1. A method for detecting methicillin resistant staphylococcal infections, said method comprising:
 - a) performing the polymerase chain reaction on clinical samples suspected of staphylococcal infection, said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high GC content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
 - b) analyzing the reaction product of step a.
2. The method of Claim 1 wherein the GC content of the DNA primers is approximately 50% or greater.
3. The method of Claim 2 wherein the sequences of the DNA primers are nucleotides 141-160 of SEQ ID NO:1 and the inverse complement of nucleotides 1929-1952 of SEQ ID NO:1.
4. The method of Claim 1 wherein an additional subsequent polymerase chain reaction is performed using DNA primers interior to the DNA primers used in the initial polymerase chain reaction.

5. The method of Claim 4 wherein the sequences of the interior DNA primers are nucleotides 568-593 of SEQ ID NO:1 and the inverse complement of nucleotides 1647-1670 of SEQ ID NO:1.
6. The method of Claim 1 wherein the sequence of the DNA primers is comprised by the coding and non-coding strands of a gene selected from a group consisting of Staphylococcus aureus and Staphylococcus epidermidis mecA genes.
7. The method of Claim 6 wherein the gene is selected from a group consisting of the S. aureus 27R, S. aureus BB270, S. aureus TK784, and the S. epidermidis WT55 mecA genes.
8. The method of Claim 1 wherein the method is used to detect methicillin resistant staphylococci selected from the group consisting of S. aureus, S. epidermidis, S. haemolyticus, S. simulans, S. carnosus, and S. saprophyticus.
9. The method of Claim 1 wherein the reaction product of step a) is analyzed by gel electrophoresis.
10. A method for the rapid release of DNA from staphylococci, said method comprising:
 - a) treating a sample containing staphylococci with lysostaphin;
 - b) treating the resultant sample of step a with proteinase K; and
 - c) incubating the resultant sample of the step in a boiling water bath.
11. The method of Claim 10 wherein the sample containing staphylococci is derived from blood, urine, spinal fluid, an abscess or bacteriological growth medium.
12. A method for detecting methicillin resistant staphylococcal infections in a sample of interest, said method comprising:
 - a) treating a sample of interest with lysostaphin;
 - b) treating the resultant sample of step a with proteinase K;
 - c) incubating the resultant sample of step b in a boiling water bath;
 - d) performing the polymerase chain reaction on the resultant sample of step c) said polymerase chain reaction being primed by DNA primers, said DNA primers being composed of two oligonucleotides of high GC content, wherein one oligonucleotide has a DNA sequence comprised by the coding strand of a Staphylococcus mecA gene and the second DNA primer has a DNA sequence comprised by the non-coding strand of a Staphylococcus mecA gene; and
 - e) analyzing the reaction product of step d.
13. The method of Claim 12 wherein the GC content of the DNA primers is approximately 50% or greater.
14. The method of Claim 13 wherein the sequences of the DNA primers are nucleotides 141-160 of SEQ ID NO:1 and the inverse complement of nucleotides 1929-1952 of SEQ ID NO:1.
15. The method of Claim 14 wherein an additional subsequent polymerase chain reaction is performed using DNA primers interior to the DNA primers used in the initial polymerase chain reaction.
16. The method of Claim 15 wherein the sequences of the interior DNA primers are nucleotides 568-593 of SEQ ID NO:1 and the inverse complement of nucleotides 1647-1670 of SEQ ID NO:1.
17. The method of Claim 12 wherein the sequence of the DNA primers is comprised by the coding and non-coding strands of a gene selected from a group consisting of Staphylococcus aureus and Staphylococcus epidermidis mecA genes.
18. The method of Claim 17 wherein the sequence of the DNA primers is comprised by the coding and non-coding stands of a gene selected from a group consisting of the S. aureus 27R, S. aureus BB270, S. aureus TK784, and the S. epidermis WT55 mecA genes.
19. The method of Claim 12 wherein the method is used to detect methicillin-resistant staphylococci selected from the group consisting of S. aureus, S. epidermidis, S. haemolyticus, S. simulans, S. carnosus, and S. saprophyticus.
20. The method of Claim 12 wherein the reaction product of step d) is analyzed by gel electrophoresis.

21. The method of Claim 12 wherein the sample of interest is derived from blood, urine, spinal fluid, an abscess or bacteriological growth medium.

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Application Number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 92307307.6
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P, A	WO - A - 92/05 281 (CHUGAI SEIYAKU KABUSHIKI KAISHA) * Abstract: claims 3-13, 16 *	1, 3, 5, 12, 14, 16	C 12 Q 1/68 C 07 H 21/00 C 12 Q 1/14 /(C 12 Q 1/14 C 12 R 1:445 C 12 R 1:45)
A	WO - A - 91/08 305 (U-GENE RESEARCH B.V.) * Abstract: claims *	1, 3, 5, 12, 14, 16	
A	CHEMICAL ABSTRACTS, vol. 102, no. 15, April 15, 1985 Columbus, Ohio, USA W.F. NAUSCHUETZ et al. "Rapid detection of oxa- cillin-resistant staphylococci using an auto- mated system." page 335, abstract-no. 128 675r & J. Med. Technol 1985, 2(1), 60-2		TECHNICAL FIELDS SEARCHED (Int. Cl.5)
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The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 10-11-1992	Examiner SCHNASS
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			



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Application Number

-2-

EP 92307307.6

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
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			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
The present search report has been drawn up for all claims			
Place of search VIENNA	Date of completion of the search 10-11-1992	Examiner SCHNASS	
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	